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(54) **Use of 5-(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxy-phenyl)-2-isopropylvaleronitrile.**

(57) 5-((3,4-dimethoxyphenetyl)methylamino)-2-(3,4-dimethoxy-phenyl)-2-isopropylvaleronitrile or a pharmaceutically acceptable salt thereof is effective to prevent metastasis of cancer.

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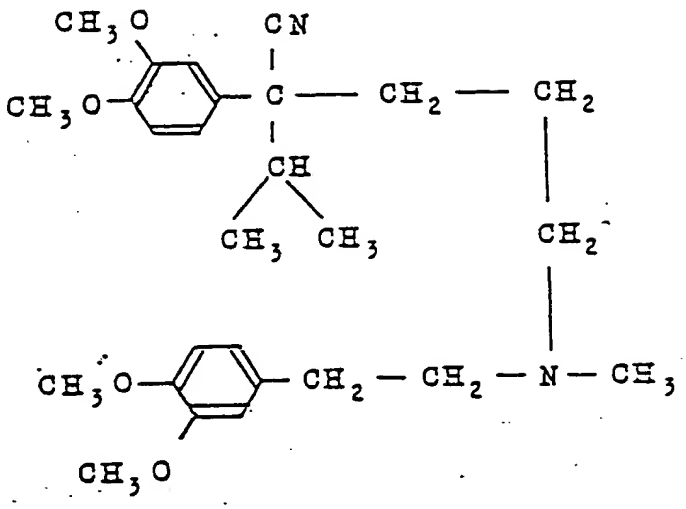
Use of 5-((3,4-dimethoxyphenethyl)methylamino)-2-(3,4-dimethoxy-phenyl)-2-isopropylvaleronitrile

This invention relates to a novel agent for preventing metastasis of cancer, i.e. an anti-metastatic agent.

More particularly it relates to an anti-metastatic agent comprising 5-[(3,4-dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropyl-

valeronitrile of the formula



or a salt thereof as an active ingredient.

In the recent statistics of the survey, cancer has occupied the first place in death causes in Japan, instead of cerebrovascular diseases. 24% of deaths, i.e. one among four, died of cancer. This mortal disease causes indescribable pain not only to the body but to the mind of a patient. In addition, cancer would most frequently attack those in the prime of life (i.e. in fourties to fifties) and playing important roles both in society and in their own homes so that their families also suffer from serious mental and economical damages.

Therefore various studies to reveal the fundamental cause of cancer and to establish epoch-making processes for the treatment and diagnosis thereof

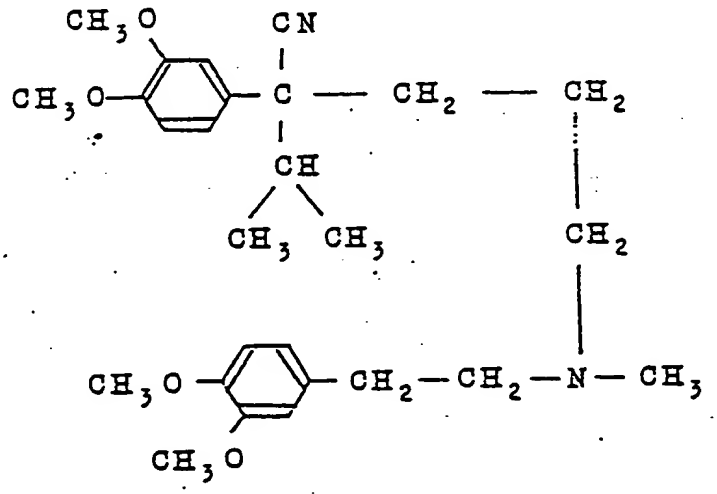
have been carried out all over the world to thereby gain ascendancy over cancer step by step. These studies have brought about significantly improved treatments and diagnosis of cancer, so that it can be completely cured in most cases if detected early enough.

Even if the original tumor is completely removed by early diagnosis followed by a surgical operation, however, tumor cells would metastasize to another organ at the time of the diagnosis in more than half cases. That is, many patients died of metastasis of cancer. Accordingly it is one of the most important problems in the treatment of cancer to prevent its metastasis.

Metastasis, which is a specific, complicated and important characteristic of cancer, would comprise many steps such as liberation of cancerous cells from the primary portion, transfer via blood or lymph vessels, adhesion to a blood or lymph vessel of an organ, infiltration and growth. The metastasis of cancer is an important factor governing the recuperation of a patient. However studies thereon still remain significantly backward since appropriate experimental system to evaluate the metastasis is quite limited. The mechanism of metastasis has not been clarified up and few countermeasures have been established at present.

In order to lower the mortality from cancer, it is a very important problem to prevent and treat the metastasis. Few antimetastatic agents, however, have been known to date.

Under these circumstances, we have tried to develop an agent for preventing metastasis of cancer, i.e. an antimetastatic agent, for a long time and found that verapamil, i.e. 5-[(3,4-dimethoxyphenethyl)methyl]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitrile of the following formula or its salt such as hydrochloride would be unexpectedly effective as an antimetastatic agent.



Accordingly it is an object of the present invention to provide a novel antimetastatic agent.

Verapamil of the above formula has been used as a therapeutic agent for ischemic cardiac diseases

in treating, e.g., stenocardia, coronary arteriosclerosis (chronic ischemic cardiac diseases, silent ischemic cardiac diseases and arteriosclerosis cardiac diseases), and myocardial infraction.

Verapamil hydrochloride has a melting point of 138.5 to 140.5°C (decomp.).

To further illustrate the present invention, the following examples will be given.

Example 1

Effect of verapamil on plumonary metastasis of B16 melanoma BL-6

B16 melanoma BL-6, isolated by Dr. Hart et al. in U.S.A., is a cell line which infiltrates through a bladder membrane and shows metastatic potential. 5×10^4 cells of B16 melanoma BL-6 were inoculated into the tail vein of a male C57BL/6J mouse. Verapamil hydrochloride was administered intraperitoneally once a day two days before the inoculation of tumor cells and three days thereafter, that is, six times in total. On the 25th day of the transplantation, the mouse was anatomized to observe the metastasis to the lungs. The degree of the metastasis was evaluated by the number of plumonary nodules. The evaluated values were represented by range, median and mean + DS. a mark "a" indicates that a significant difference has been observed when compared with a control, that is, p is smaller than 0.05 (Student's t-test). Ten mice were used per a group. Table 1 shows results.

Table 1

Dose of verapamil hydrochloride (mg/kg)	No. of pulmonary nodules					
	Median	Range	% to control	Mean ± SD	% to control	Significant Difference
30	4.5	3∿ 9	32	5.0±1.7	29	a
40	12	1∿19	86	9.8±6.8	56	a
50	9.5	2∿13	68	8.0±4.3	46	a
Control	14	4∿33	100	17.5±9.3	100	

Example 2: Metastasis of B16 melanoma BL-6 to lungs and lymphonodi

25×10^4 cells of B16 melanoma BL-6 were transplanted to the right forefoot of a C57BL/6J male mouse. Cancerous cells would spantaneously metastasize to the right nodi lymphatic axillares and lungs with the elapse of time. Verapamil hydrochloride was intraperitoneally administered once a day from the fifth to 16th day (i.e. 11 times) after the transplantation of the cancerous cells. On the 17th day of the transplantation, the right forefoot including the primary tumor was cut off. On the 38th day the mouse was anatomized to determine the number of plumonary nodules.

Table 2 shows the result.

Table 2

Dose of verapamil hydrochloride (mg/kg)	No. of pulmonary nodules					
	Median	Range	% to control	Mean ± SD	% to control	Significant Difference
30	11	3∿21	147	12.3± 6.5	75	
40	5	1∿12 [a]	67	5.8± 3.7	35	
50	1.5	0∿12	20	3.5± 4.3	21	a
Control	7.5	1∿42	100	16.5±17.1	100	

Example 3

Effect of verapamil on plumonary metastasis of highly metastatic clone NL-17 of mouse colonic cancer colon 26 adenocarcinome

5×10^4 cells of a highly metastatic cell strain clone NL-17 were transplanted into a vein of a BALB/C femal mouse. Verapamil hydrochloride was administered intraperitoneally to the mouse once a day two days before the inoculation of tumor cells and three days thereafter, i.e. six times in total. On the 23th day of inoculation, the mouse was anatomized to determine the number of metastatic pulmonary nodules. Results are shown in Table 3.

Table 3

Dose of verapamil hydrochloride (mg/kg)	No. of pulmonary nodules					
	Median	Range	% to control	Mean ± SD	% to control	Significant Difference
60	1	0∿96	2.1	13.1±31.3	15.6	a
75	1.5	0∿67	3.2	18.6±24.5	22.1	a
Control	47.5	5∿>200	100	84 ±71.3	100	

Example 4

Effect of verapamil on plumonary metastasis of highly metastatic clone NL-22 of mouse colonic cancer colon 26 adnocarcinome

1×10^6 cells of a highly metastatic cell strain NL-22 of mouse colonic cancer colon 26 were transplanted into the right forefoot of a BALB/C female mouse. Cancerous cells would spontaneously metastasize to the lungs with the elapse of time. Verapamil hydrochloride was intraperitoneally administered to the mouse once a day from the sixth to 12th day of the transplantation, i.e. six times in total. On the 13th day of the transplantation, the right forefoot including the primary carcinoma was cut off. On the 29th day, the mouse was anatomized to determine the number of plumonary nodules.

Table 4

Dose of verapamil hydrochloride (mg/kg)	No. of pulmonary nodules					
	Median	Range	% to control	Mean ± SD	% to control	Significant Difference
50	34.5	12∿ 55	91	33.1±14.6	59	
60	22.5	10∿ 41	59	23.5± 8.5	42	a
75	19	7∿ 74	50	27.8±21.8	50	a
Control	38	22∿126	100	55.9±31.8	100	

Examples 1 to 4 as shown above clearly indicate that the verapamil hydrochloride according to the present invention remarkably prevents metastasis of cancer not only in a single experimental system but also in various experimental systems for cancer metastasis in animals.

Accordingly the verapamil according to the present invention is useful as an excellent agent for preventing metastasis of cancer, i.e. an anti-metastatic agent.

The dose of the verapamil of the present invention as an antimetastatic agent depends on various factors such as the type of cancer and the condition of the patient. It may be usually administered to an adult orally or parenterally in a dose of 10 to 500 mg once to four times a day without any limitation.

It may be formulated into various forms such as powder, grain, granule, tablet, capsule and injection. Formulation may be carried out in a conventional manner with the use of conventional carriers.

In addition to the use as a therapeutic agent administered to cancerous patients, the verapamil of the present invention is further available in

preventing metastasis in those who have received medical treatments such as chemotheraoy, endocrinotherapy and immunotherapy, radiotherapy or surgical treatments.

Needless to say, the agent of the present invention may be simultaneously administered with other carcinostatic agents.

Toxicity of the verapamil hydrochloride as used in the present invention will now be shown.

Acute toxicity

Table 5 shows LD_{50} (mg/kg) thereof.

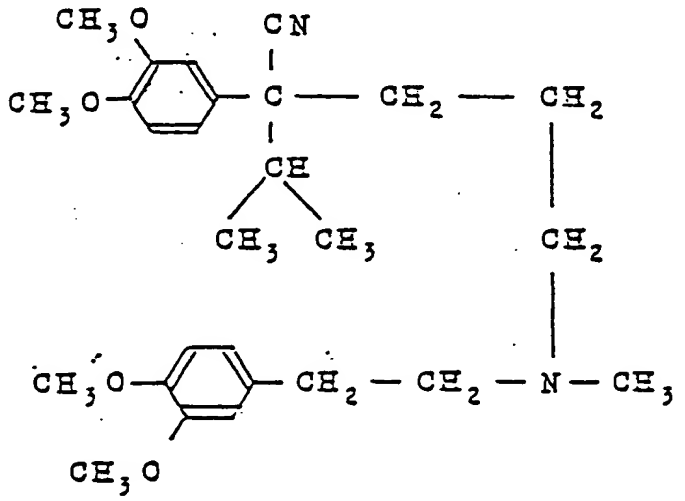
Table 5

Animal	Sex	Oral	Subcutaneous	Intramuscular	Intravenous
Mouse	Male	163	68	-	7.6
Rat	Male	108	107	118	16
	Female	126	-	-	-
Dog	Male and female	>400	-	25	-

As described above in detail, the verapamil of the present invention is remarkably effective as an antimetastatic agent. Since metastasis is the cause of deaths due to cancer in most cases, the present invention is extremely valuable.

CLAIM:

Use of 5-((3,4-dimethoxyphenetyl)methylamino)-2-(3,4-dimethoxy-phenyl)-2-isopropylvaleronitrile of the formula given below or a pharmaceutically acceptable salt thereof for the preparing of a composition for prevention of metastasis of cancer.



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EUROPEAN SEARCH REPORT

Application number

EP 85 10 4787

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
E	EP-A-0 123 850 (THE BOARD OF GOVERNORS OF WAYNE STATE UNIVERSITY) * Claims 1,7; page 1, line 12 - page 9, line 32 * -----	1	A 61 K 31/275

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 61 K 31/00

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	01-07-1985	THEUNS H.G.

CATEGORY OF CITED DOCUMENTS

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E : earlier patent document, but published on, or after the filing date
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